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Annual Progress Report

M. L. Wolbarsht
M. A. Orr
B. S.Yamanashi
J. S. Zigler
I.B.C. Matheson

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D. C. 20314

Contract DAMD 17-74-C-4133

Duke University Medical Center
Durham, North Carolina 27710

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LIST OF TABLES AND FIGURES

SUMMARY

Changes in lens proteins following exposure to IR irradiation have been used to detect the earliest changes possible in cataract formation. Rat lenses maintained in organ culture have been used to determine the temperature which will initiate cataractogenesis. Exposures from a CW neodymium laser (1060 nm) were used to determine what energy levels are necessary to produce protein changes. These changes have been characterized by thin layer isoelectric focused electrophoresis and sodium dodecyl sulfate electrophoresis.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences—National Research Council.

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INTRODUCTION

The purpose of this work was to investigate the mechanism of formation of lenticular cataracts following IR irradiation from a CW neodymium (1060 nm) source. The experiments were designed to determine the specific initial interaction of laser radiation in the infrared region with the lens that leads to cataract formation.

Little is known about the mechanism of cataract formation following acute exposure to near infrared radiation from lasers. However, individuals exposed for years to infrared rays from molten glass or high-temperature ovens develop what is known as heat, glassblowers', or glassworkers' cataract (20) , and it is likely there are some similarities. Lenticular changes occurring with chronic exposures to high-temperature ovens start with a cobweb-like opacity in the posterior cortex. The opacity increases in size and density and develops into a saucer-shaped posterior cataract. The posterior opacity continues to grow and ultimately forms a complete opacity resembling a senile cataract (9). A very similar appearing cataract can be produced in the rabbit by repeated exposure to infrared rays from a carbon arc light. When the rays are selectively filtered it is the wavelengths between 800 and 1200 nm that produce the lenticular changes (12).

The actual mechanism of cataract formation from infrared irradiation, thermal or otherwise, is still undefined. Earlier works conclude that the light and infrared rays are absorbed at the pigment epithelium of the iris and converted to heat, that the heat damages the adjacent epithelium of the lens, and that a cataract develops later as a result of this epithelial damage (12). Another study concludes that some of the infrared energy reaching the lens is absorbed, warming the lens. It was calculated the lens of a dilated human eye can increase 9°C. in temperature by looking through a porthole about 1 foot away for 1 minute at molten glass (8). This temperature effect is especially marked at the focal point of the combined cornea-lens which is just posterior to the lens.

In an occupational setting, such acute exposures would not occur. It has been calculated that the rise of temperature in the iris and anterior portions of the lens from the energy levels of these chronic exposures is very slight, less than 1°C. In any case the thermal theory of cataractogenesis states that this increase in temperature of the anterior portion of the lens (which is in contact with the heated iris) will in time produce denaturation of a sufficient amount of lens protein to initiate a cataract. The cataract induced by infrared irradiation is thought to be similar to senile cataract in that there is a loss of ascorbic acid and a decrease in the relative amount of soluble protein.

In contrast with this theory involving heat conduction of the iris, a possibility also exists that the infrared radiation is absorbed directly by the lens. Absorption by the lens is low in the near infrared, but at 1300 nm it abruptly becomes high. At 1060 nm the absorption is approximately 20%. Since the increase in temperature in the lens during

chronic exposures is so minute, protein denaturation or inactivation of enzyme systems due to thermal stress appears unlikely. Rather it is likely that some specific chemical process is stimulated or suppressed by the absorption of the infrared radiation. Furthermore it is possible that the photochemical stress of the infrared irradiation could cause local protein damage resulting in a configurational change or the production of abnormal (miscoded) proteins. The soluble lens proteins, which include the α, β, and γ crystallins, comprise over 90% of the dry weight of the lens. The function of the lens crystallins is to produce a matrix which is fully transparent to visible light and which remains so during lens accommodation. It seems likely that precisely ordered optical interactions among the various lens proteins would be required to produce such a transparent system. Any disruption in the strict structure of the lens could lead to a measurable loss of transparency, especially since the repair processes go on at a low rate.

The possibility of a non-thermal process in initiating IR cataract formation suggests a similarity between IR cataracts and ultraviolet-induced senile or brunescent cataracts. Brunescent cataracts have been widely studied and a review of these studies has suggested programs to be used in the study on infrared laser cataractogenesis. Briefly the changes noted in brunescent (senile) cataracts are a decrease in the total lens proteins mostly in the high molecular weight moiety (4), accompanied by a loss of ascorbic acid and a buildup of glutathione and the insoluble albuminoid protein fraction (7). In a wide variety of cataracts (brunescent, X-ray, UV, glass-blowers', galactosemia-induced) the α-crystallin converts to the insoluble albuminoid form. This conversion probably involves a conformational change in the portion of the protein that masks the sulfhydral group (15) and once exposed, helps form a complex between the soluble α-crystallin and the trace amounts of glycoprotein present (6).

One possible initial step in ultraviolet cataractogenesis is the photooxidation of tryptophan as indicated by the work of Zigman (24) and Kurzel et al. (11). Tryptophan is probably the most susceptible amino acid to UV degradation. For this reason, the portion of the protein with tryptophan has the highest probability of acting as an immediate site for secondary chemical reactions such as oxidation. It has been established by several groups (15, 5) that these UV-induced cataracts have many points in common with brunescent senile cataracts.

The human lens is not normally subjected to radiation longer than 2000 nm or shorter than 293 nm, as the cornea absorbs very strongly in these regions. However, the lens absorbs most of the radiation from 300 to 400 nm and between 1100 and 1400 nm. The relative effectiveness of various portions of the near infrared as a function of wave length for cataractogenesis is not known at present.

It seems likely that within the UV sensitive range the relative quantum efficiency for cataractogenesis has a relation to the photon energy, with the higher energy photons being the more effective. Most important, however, may be any strong absorption bands in tryptophan. In brunescent cataracts, the photooxidation of tryptophan proceeds through

several degradation steps: kynurenine to 3-hydroxykynurenine and its degradation via further hydrolyzed products of kynurenine. These latter degradation products can be detected by ultraviolet fluorescence techniques (10, 11). Glycoproteins have also been implicated with senile cataract formation (2) and particularly with the glycosidase activity in lenses. This enzyme activity may be specifically related to the excited state of the glycoside of 3-hydroxykynurenine. Glycoproteins may also act as the source for "active" glucose molecules originallysuggested by Spiro (18) and found by Spector (16) as the trigger for the onset of α-crystallin aggregate formation. However, the "active" glucose binds the low molecular weight crystallin components to each other to form the larger albuminoid moieties.

Our current research program has sought to identify a similar chain of protein degradation products connected with infrared cataractogenesis. Analysis of the lens protein has been accomplished in our previous studies primarily through gel electrophoresis. The electrophoretic process, by separating the protein fractions into identifiable bands, allows us to determine which protein constituents have been affected, which have decreased in concentration and what new fractions are appearing. Some of the changes which occur in the various crystallins can be conveniently studied after they are separated by isoelectric focused thin gel electrophoresis of the homogenized lens. This technique is now widely used to separate and characterize such protein mixtures. It is basically electrophoresis in a pH gradient which separates the various proteins according to their isoelectric points. As the thin layer polyacrylamide gel can be efficiently cooled even with high current densitites, the experimental time can be markedly shortened. The exact procedure used was established by Zigler (25). Gel electrophoresis of proteins denatured to their constituent polypeptides by sodium dodecyl sulfate (SDS) is another widely used procedure. The electrophoretic mobility of such denatured proteins is linearly related to their molecular weight which makes identification of the resultant bands much easier.

PREVIOUS RESULTS

The initial portion of this program involved amino acid analysis of cataracts produced by a CW neodymium laser (1060 nm). The results showed no marked changes in the amino acid distributions as compared with a normal lens. This suggests that the chief mechanism of cataract formation is most likely protein denaturation. Further laser studies produced cataracts which were analyzed by isoelectric focused thin layer gel electrophoresis. Analysis of the cataractous portions of the lens showed an almost complete disappearance of the soluble crystallins (α, β_H, β_L, and γ). This indicates a strong linkage between opacity formation and the complete precipitation of these crystallins. In the clear portions of the cataractous lenses the concentrations of the soluble proteins were almost unchanged except for the β_H crystallin fraction which could be the earliest indication of cataractogenesis.

Concurrent experiments on the possible direct effect of heat on the lens have been conducted by incubating extracted and homogenized lenses at temperatures from 37° to 45°C. The higher temperatures showed marked diminution of the α crystallin fraction.

Results of column chromatography run on normal and cataractous lenses indicate that the disappearance of the soluble crystallins is probably by aggregation into insoluble albuminoid forms with a molecular weight greater than 1,000,000.

PRESENT PERIOD

During this period of the contract whole rat lenses maintained in organ culture for three days at a controlled temperature of 40°C showed a loss of a possible β crystallin fraction and a decrease in the α crystallin motility. Rat lenses maintained in organ culture for seven days at 38.5°C showed no change from the control. These results determined by isoelectric focused thin-layer gel electrophoresis indicates that the threshold temperature for inducing changes in the rat lens protein is between 38.5°C and 40.0°C. The disappearance of the β band due to the heat stress also fits in with a hypothesis (13) of how the transition of the soluble crystallin fraction into the insoluble albuminoid protein fraction in the rabbit lens occurs. The rat lenses were used as a preliminary test to determine the temperature which will initiate cataractogenesis. Incubation studies on rabbit lens organ cultures have begun, utilizing the information gained from the rat lens studies.

Concurrent with these experiments, exposures from a CW neodymium laser (1060 nm) were used to determine what energy levels were necessary to produce protein changes in the rabbit lens. (The energy level in joules of each exposure was determined by multiplying the power level times the exposure time.) At a power level of 2.00 W., an exposure of 50 sec. (100 joules) produced an opacity over the entire anterior surface of the lens. A lower power level (1.75W) and an increased exposure (90 sec.) (157.5 joules) produced the same result whereas a power level of 1.75 W at 50 sec. (87.5 joules) produced only a peripheral opacity. Analysis of these lenses using sodium dodecyl sulfate (SDS) gel electrophoresis, and isoelectric focused thin layer electrophoresis with and without urea showed protein changes occurring in lenses exposed to energy levels of 100 joules or more. In order to determine if protein changes can be induced in the lens without producing an opacity, rabbits will be exposed to much lower intensities for longer amounts of time.

EXPERIMENTAL METHODS

Details of Lens Incubation Studies

Lenses from 3-6 week old rats were used in all experiments. The animals were killed with ether and the eyes removed immediately. Under 10X magnification, the eye was opened posteriorly by severing the optic nerve at its entrance to the globe. From this foramen, small eye

scissors were used to cut through the sclera, choroid, and retina along one meridian forward to the limbus. The incision was extended perilimbally in a plane through the ciliary processes, the root of the iris, and the peripheral cornea. The lens was then gently rolled free of the adherent tissue taking care to prevent the dissection instruments from touching the lens. Each lens was immediately transferred to a small culture dish (Falcon, 35 by 10 mm) containing 4 ml. of medium. The culture medium employed is a mixture of 50 parts of TC 199, 20.5 parts of bicarbonate buffer (14), and 9.5 parts of a solution containing sufficient glucose, fructose, and calcium chloride to achieve final concentrations of 5 mM, 30 mM, and 1.5 mM, respectively (20). The lenses were incubated in an atmosphere of 95 percent air and 5 percent carbon dioxide at temperature of 36.5°, 37.5°, 38.5°, and 40.0°C. The culture medium was changed every other day and a sample lens removed and frozen in distilled water every day of the incubation. Each rat lens was prepared for analysis by being homogenized in 0.5 ml H_2O and centrifuged at 9,000 rpm. The supernatant containing the water soluble lens protein was saved for analysis by gel electrophoresis.

Details of In Vivo Laser Exposures

All laser exposures were made with a CW neodymium-HAG laser (Holobeam Model 250). It is a multi-mode CW laser with the majority of its output in a 1.065 μm beam, approximately 3 mm in diameter at the exit port. The beam was enlarged in collimated form to 24 mm by means of an 8X beam expander (Edmund Scientific). In a previous report (24) the laser output is shown as a function of the lamp input power to indicate the reproducibility of the output as a function of the input power. The power input was measured by a Scientech (Boulder, Colorado, Model 360) disc calorimeter which sampled the back beam of the laser. The ratio of the front to back beam of the laser output was measured periodically so that the front beam output could be accurately calculated by measuring the back beam power with an appropriate correction factor. The disc calorimeter was calibrated absolutely by passing a known current through a built-in heating resistor noting the calorimeter output. The measurements of laser power were relatively unaffected by the back reflection from the beam expander which had a constant 10% total air-glass reflection from all the surfaces.

The relative position of the eye of the exposed rabbit to the laser is the same as used previously (24). The rabbits weighing 2-3 lbs. were pigmented, from a mixed litter and 8 weeks old. They were anesthetized with 0.6 cc. sodium pentobarbitol before exposure and the eyes were left undilated. Power levels from 0.61 W to 2.00W were used for the exposure times seen in Table I.

TABLE I

Power Levels and Exposure Times for In Vivo Laser Study

Watts	Seconds	Joules
0.61	180	109.8
0.94	30	28.2
0.94	60	56.4
0.94	180	169.2
1.10	60	66.0
1.10	180	198.0
1.30	60	78.0
1.30	120	156.0
1.50	25	37.5
1.50	60	90.0
1.50	90	135.0
1.75	50	87.5
1.75	90	157.5
2.00	25	50.0
2.00	50	100.0

Following irradiation, the animal was sacrificed, the eyes removed and the lens extracted. Each lens was homogenized in 3.5 ml. H_2O, centrifuged at 9,000 rpm and the supernatant containing the water soluble lens protein saved for analysis by gel electrophoresis.

Gel Electrophoresis

In this procedure the excised lenses were placed in cold distilled water, and immediately homogenized with a Willems Polytron (Brinkman Instruments). All insoluble material was removed from the lens homogenates by centrifugation at 27.00 g for 20 minutes at 4°C. The LKB 2117 Multiphor (LKB Instruments Inc.) was used for isoelectric focused thin layer gel electrophoresis on polyacrylamide slab gels. Special, narrow range gels (PH 5-8) were prepared by the procedure recommended by LKB and were photopolymerized. For a broader spectrum, wide range gels (pH 3.5 - 10) were used, generally of the preformed types (LKB Pag Plates). Lens samples (approximately 2 mg/ml concentration) from small applicator strips of Whatman 3 MM filter paper were applied to the surface of the gel midway between the anode and cathode. Initially the amperage was set at 50 mA. As the current dropped during focusing, the voltage was increased, but the maximum power was held below 30 W. Between one and one-half to three hours the current stabilized, signaling equilibrium conditions. After an additional 20 minutes to allow the slower moving proteins to complete migration to their isoelectric points, the run was halted.

The gel was removed and stained directly for 20 minutes at 60°C with a standard staining mixture: 75 ml methanol, 155 ml water, 0.25 g Coomassie Brilliant Blue R, 7.5 g sulfosalicylic acid, and 25 g trichloroacetic acid. The plate was then destained overnight at room temperature in a

solution containing 1950 ml water, 750 ml ethanol and 240 ml glacial acetic acid. The initial standardization of the technique used frozen calf eyes from Pel-Freeze Biologicals, Inc. All test lenses were analyzed by the same procedure.

Urea-Polyacylamide Electrophresis

Gels with a final concentration of approximately 6M urea were prepared from stock solutions identical to those used for standard thin-layer gels except that all stock solutions were made up in freshly deionized 8M urea instead of water. The same stock electrophoresis buffer (tris-glycine, pH 8.3) was also used. However, in this case one part stock buffer was diluted with three parts 8M urea. Dithiothreitol was added to the diluted buffer to a concentration of 100 miligrams per liter. Deionization of 8M urea was accomplished by passing the 8M urea over a column (3 X 35cm) of the mixed resin AG 501-X8 (Bio Rad Laboratories). The electrophoretic apparatus and the mechanical techniques in preparing and handling the gels and sample solutions were identical to those used for standard thin-layer gels, except that sample protein solutions were prepared in the urea electrophoresis buffer described above. Gels were stained in Coomassie brilliant blue R, prepared by a one to twenty dilution in 5% sulfosalicylic acid -5% TCA solution of one percent aqueous Coomassie blue.

Electrophoresis in Sodium Dodecyl Sulfate (SDS)

Electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed according to the method of Weber and Osborn (23) with certain modifications. It has been demonstrated that SDS, which is an anionic detergent, denatures proteins to their constituent polypeptides and that the electrophoretic mobility of SDS-denatured polypeptides is linearly related to molecular weight. SDS was obtained from Fisher Scientific Company and was used without further purification. The gel buffer was prepared as follows: 3.9 grams $NaH_2PO_4.H_2O$, 19.3 grams $Na_2HPO_4.H_2O$, and 2.0 grams SDS dissolved in water to a volume of one liter. Before use, the gel buffer was diluted by addition of an equal volume of water. A stock solution for 10% acrylamide gels was prepared by dissolving 22.2 grams of acrylamide and 0.6 grams methylene bisacrylamide in water to give 100 milliliters of solution. This stock solution was stored at $5^{o}C$ in a brown bottle. Staining solution was prepared by dissolving 1.25 grams Coomassie brilliant blue in a mixture of 454 milliliters of 50% methanol and 46 milliliters of glacial acetic acid. The destaining solution contained 75 milliliters of glacial acetic acid, 50 milliliters of methanol and 875 milliliters of water. To prepare twelve SDS gels, 15 milliliters of undiluted gel buffer, 13.5 milliliters of acrylamide stock solution, 1.5 milliliters ammonium persulfate solution (10 milligrams per milliliter) and 0.045 milliliters TEMED were mixed and then pipetted into the gel tubes, five millimeters in internal diameter and approximately fourteen centimeters in length.

Samples were prepared by adjusting the protein concentrations to approximately one milligram per milliliter in 0.01M sodium phosphate buffer, pH 7.2, 1% in SDS, and 1% in 2-mercaptoethanol. Before applying

samples to the gels they were placed in a boiling water bath for several minutes to insure complete denaturation. Samples were applied to the gels as outlined above and were electrophoresed at 4.5 milliamperes per gel for four to six hours. After completion of the run the gels were removed from the tubes and cut through the bromophenol blue tracking band. The bottom parts of the gels were discarded and the top sections were left overnight in the following solution: 227 milliliters methanol, 227 milliliters water, and 46 milliliters glacial acetic acid. This solution is identical to the staining solution except that the Coomassie blue is omitted. This step was added to the procedure to leach out unbound SDS from the gels in order to facilitate staining. The gels were then transferred to tubes containing staining solution for approximately forty-five minutes after which they were rinsed and placed in destaining solution in a 37°C oven. Destaining was complete after several changes of destaining solution.

EXPERIMENTAL RESULTS FROM PRESENT PERIOD: DETAILS AND DISCUSSION

Incubated Lenses

Because the purpose of this study is the early detection of cataracts caused by IR irradiation, we hoped to study the changes in the incubated lenses before any actual opacities began to form. Lenses from older rats (10 weeks) appeared less susceptible to heat stress than the younger lenses (4-6 weeks). Younger lenses therefore were used because of the ease of cataract formation. The 4-6 week old rat lens maintained its transparency for up to 13 days at 34.5°C., 8 days at 37.5°C., 7 days at 38.5°C., and only 3 days at 40.0°C. In each case, only completely clear lenses were analyzed.

Fig. 1 shows the thin-layer gel (pH 5-8) on which samples of the lens proteins incubated at various temperatures have been isoelectric focused.

Figure 1. Thin-layer acrylamide gel (pH5-8) showing protein bands from rat lens incubated at different temperatures. Arrow indicates missing band assumed to represent β - crystallin.

The obvious loss of the band in the 40° sample suggests that the threshold temperature for causing changes in the lens proteins is between 38.5° and 40°C. A similar result is seen in a gel made up at a broader pH (pH 3.5-10). Again there is the loss of a band which is thought to represent a β crystallin.

In Vivo Laser Exposed Lenses

Each laser exposure used on a rabbit's eye in this study caused some degree of cloudiness in the lens. The energy levels below 90.0 joules (1.50W, 60 sec.) produced primarily peripheral opacities beginning where the ciliary muscles attach to the lens capsule, whereas every lens exposed to energy levels of 100 joules (2.00W, 50 sec.) or more developed a solid opacity over the entire anterior surface of the lens.

Electrophoretic analysis of typical exposures are pictured in Figs. 2, 3, and 4 which show the separated protein bands of exposed and control lenses by isoelectric focusing using urea, and non-urea gels and SDS gel electrophoresis. Fig. 2 (a thin-layer acrylamide gel, pH 3.5-10) shows a decrease in the α crystallin in the samples irradiated at higher energy levels i.e. 100 joules (2.00W, 50 sec.), 157.5 joules (1.75W, 90 sec.). Our earlier studies using lens homogenates showed the same loss of α-crystallin and the creation of a high molecular weight crystallin detected by column chromatography. There are studies (16, 17) which report a high-molecular weight protein fraction with molecular weight above 10,000 Daltons in calf lens which turned out to be composed of α- crystallin polypeptides.

Figure 3, showing an analysis of the samples, using a 6M urea gel, exhibits a loss of a distinct band in the high energy exposed samples, i.e. 135.0 joules (1.50W, 90 sec.), 157.5 joules (1.75W, 90 sec.), 87.5 (1.75W, 50 sec.), 100 joules (2.00W, 50 sec.). Definite identification of this band is still to be determined. In Figure 4 (SDS acrylamide gel) the gradual loss of band 7 in the soluble fraction with increasing energy level of laser exposure is comparable with the gradual appearance of band 7 in the insoluble fraction. Band 7 corresponds with the band produced when purified α-crystallin is run on SDS gels. This is evidence of a transition of soluble to insoluble protein.

DISCUSSION

Our earlier work with incubated calf lens homogenates and purified lens crystallins using column chromatography indicated that the α-crystallin had shifted to a slightly higher molecular weight. The very high molecular weight aggregate formed from the purified α-crystallin incubated at 40° was much less than that formed from the whole lens homogenates. This indicates that traces of the β or γ crystallins may assist in the formation of the aggregate. This hypothesis is supported by Liem-The and Hoenders (13) in their study on the formation of the water insoluble rabbit lens proteins. They show a similarity in the subunit composition of α-, HM-crystallin (high molecular weight crystallin) and a urea soluble fraction isolated

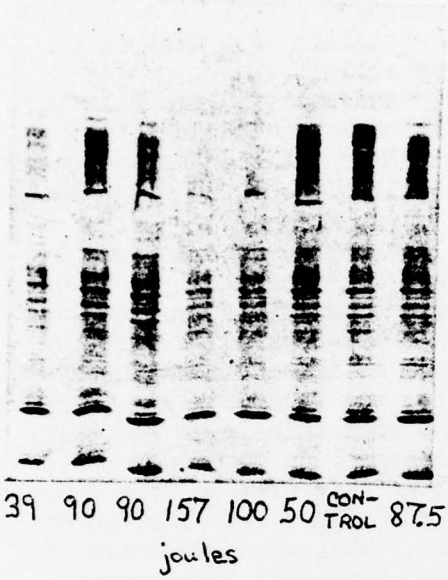

Figure 2. Thin-layer acrylamide gel showing laser exposed rabbit lens protein.

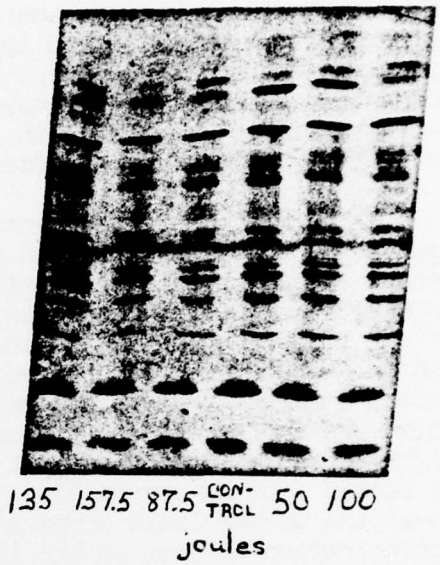

Figure 3. Thin-layer urea gel showing laser exposed rabbit lens protein.

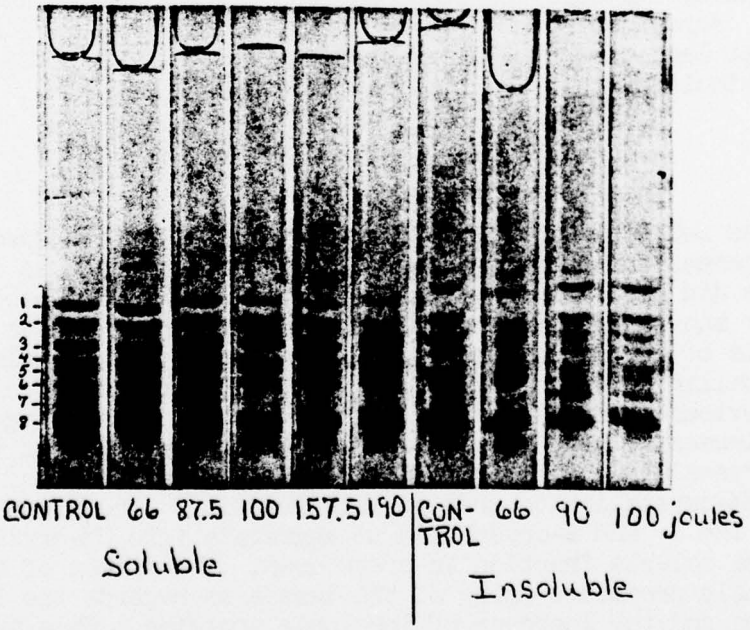

Figure 4. SDS gels showing laser exposed rabbit lens protein.

from the rabbit lens. In addition they have determined that in going from α-crystallin via HM-crystallin to the urea soluble fraction, an increasing amount of β-crystallin chains are involved. They hypothesize that the α-and β- crystallin molecules are arranged either in complexes of α-crystallin molecules and aggregated β-crystallin or a mixture of aggregated α-crystallin and aggregated β-crystallin.
As suggested earlier by Spector et al (17), van Kamp (21) and Stauffer, Rothschild, Wandel and Spector (19) a transition may take place from α-crystallin to HM-crystallin. In this light HM-crystallin may be regarded as an intermediate in the process of insolubilization of the crystallins.

Throughout the experiments using rat lens homogenates and whole lens organ cultures incubated at a temperature of 40° as well as rabbit lenses exposed to a CW neodymium laser (1060 nm), analysis by thin-layer isoelectric focusing has shown a decrease in the α-crystallin fraction of the soluble protein. In the clear portions of lenses in which exposure from a CW neodymium laser produced an opacity, the β_H crystallin fraction showed a markedly decreased motility. In the urea thin layer gel (Fig. 6) there are indications that a urea soluble component is dropping out in the higher irradiated samples. It could be that if this urea-soluble compound is formed from HM-crystallin, the stress of the high energy impingement could cause the urea-soluble compound to break down. This would decrease its concentration as seen in the 135 joule (1.50W, 90 sec.), 157.5 joules (1.75W, 90 sec.), 87.5 joules (1.75W, 50 sec.) and 100 joules (2.00W, 50 sec.) samples in Fig. 3 and possibly account for part of the increase in the HM-crystallin. The loss of band 7 (Fig. 8) in the soluble fraction and the increase of band 7 in the insoluble fraction is evidence of a transition of protein from the soluble to the insoluble form as a result of laser exposure. The action of laser exposure can be considered a premature or accelerated aging process. For example an early study by Dische et al. (7) shows that in the aging rat lens the crystallin fraction decreases directly as the insoluble albuminoid protein fraction increases.

CONCLUSIONS

Rat lenses subjected in vitro to a 40°C. temperature showed a loss of β crystallin whereas the lenses subjected to lower temperatures for longer periods of time did not. Rabbit lenses exposed in vivo to a neodymium (1060 nm) laser showed protein changes occurring in those lenses exposed to energy levels of 100 joules or more. The higher energy levels caused a loss of α-crystallin as well as the loss of a distinct band of urea soluble protein. A previous study (24) reports the formation of a HM-aggregate in heat stressed lenses. These results fit in with the hypothesis (11) that the α- and β-crystallin chains are involved in the transition of the α-crystallin to HM-crystallin to urea soluble fraction. The IR irradiation may be causing the α- and β-crystallin to aggregate into HM-crystallin as well as the urea soluble fraction to break down. The effect of the laser exposures resemble premature aging of the lenses as regards the loss of soluble proteins and the increase of insoluble proteins. This hypothesis is speculative and requires further study.

EXPERIMENTS IN PROGRESS AND POSSIBLE LINES OF FUTURE INVESTIGATION

Three major lines of investigation will be pursued in the coming period. One is the exposure of rabbit lenses *in vivo* to a neodymium laser (1060 nm) at lower intensities than previously utilized and for longer exposure periods in order to study the lenses before cataracts are formed. The second group of experiments will involve incubation of rabbit lenses *in vitro*. The third group will entail exposing rabbit lenses *in vivo* to a heat lamp. This chronic exposure will more closely simulate the occupational situation which induces the heat cataract.

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